Structure
Previews

A Zinc Lock on GGDEF Domain Dimerization Inhibits *E. coli* Biofilms

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http://dx.doi.org/10.1016/j.str.2013.06.011

In this issue of *Structure*, Zähringer and colleagues present crystal structures of an important signal transduction enzyme, diguanylate cyclase DgcZ from *E. coli*. The authors show that zinc ions bound to the CZB domain inhibit enzyme activity and reveal how zinc availability affects biofilm formation.

In the world of bacterial signal transduction, it is a rare treat to see a study that identifies a signaling molecule, uncovers how it changes protein conformation and activity, and explores the biological context for small-molecule regulation. In general, the transduction aspect of signal transduction in bacteria is reasonably well understood, whereas knowledge of sensory mechanisms lags far behind. The situation with diguanylate cyclases (DGCs), a class of enzymes involved in the synthesis of the ubiquitous bacterial second messenger, cyclic dimeric GMP (c-di-GMP), is no exception. We know relatively little about the signals that turn DGC activities on and off. DGCs function as homodimers, making c-di-GMP from two molecules of GTP in a manner in which each enzyme subunit contributes one GTP substrate to the catalytic site at the subunit interface (Chan et al., 2004; Ryjenkov et al., 2005). Cyclic di-GMP is a molecule that controls a broad range of physiological parameters in diverse bacteria (reviewed in Römling et al., 2013). In the proteobacteria, where it has been studied the most, c-di-GMP is best known for regulating a lifestyle switch from a motile, single-cellular state to a sessile state prone to formation of multicellular biofilms.

DGCs are among the most abundant signal transduction proteins. The PFam domain database lists >42,000 GGDEF domains from all sequenced species (March 2013; Punta et al., 2012), the majority of which possess DGC activity. (For comparison, catalytic domains of all adenylate and guanylate cyclases make up about one fourth of that number.) Many bacterial species contain dozens of DGCs that affect various cellular targets through unique or overlapping c-di-GMP-dependent networks (Römling et al., 2013). How do tiny bacterial cells cope with the potential "regulatory nightmare" of managing numerous DGCs? One critical mechanism is to rely on environmental and intracellular signals to turn activities of relevant DGCs on and off in appropriate situations. Several such signals directly affecting DGC activities have been identified, including oxygen, NO, and red and blue light. A fraction of DGCs are regulated by phosphorylation of the receiver (REC) domains of two-component response regulators covalently linked to the GGDEF domains. These REC domains are phosphorylated by sensory histidine kinases. However at present, the majority of signals affecting DGCs remain unknown (Römling et al., 2013).

In this issue of *Structure*, Zähringer et al. (2013) revealed a new signal controlling DGC activities as zinc. The authors solved crystal structures of the DGC DgcZ (formerly YdeH) from *E. coli* (at 3.9 Å resolution) as well as the structures of its CZB and GGDEF domains (at 2.2 and 1.8 Å resolution), where CZB is a recently described Zn^{2+}-binding domain (Draper et al., 2011). They observed that the CZB domains provide a homodimerization interface that brings two GGDEF domains together. Zn^{2+} binds to the 3His-Cys motif of the CZB domain with subfemtomolar affinity. It appears that Zn^{2+} binding fixes the GGDEF domains of a homodimer in a misaligned, nonproductive conformation. Therefore, Zn^{2+} functions as a lock that disallows catalysis. Because structures of a catalytically active DGC do not exist, the authors modeled such a structure to fit the proper alignment of two GTP substrates in the catalytic site. Based on this model, it appears that the transition from the nonproductive to productive homodimeric GGDEF conformation involves a relative movement of the GGDEF domains by ~6 Å. The authors suggest that Zn^{2+} removal should restore catalytic activity. Consistent with the zinc lock model, addition of the Zn^{2+}-chelating agent EDTA to the fully Zn^{2+}-loaded DgcZ increased enzyme turnover number in vitro by ~10-fold without affecting the homodimeric state of DgcZ.

DgcZ belongs to a group of >300 proteins with the CZB-GGDEF domain architecture, mostly from *E. coli*, but also from other γ-, β-, and α-proteobacteria, some as exotic as symbionts of deep sea vent worms. Our analysis of protein domains, directly linked to CZB, revealed that >60 proteins (PFam database) from diverse bacteria have CZB linked to EAL domains, which possess c-di-GMP phosphodiesterase activity (Römling et al., 2013). Clearly, monitoring Zn^{2+} availability to adjust c-di-GMP-dependent regulation makes sense to a variety of bacteria, and they may do so either via regulating c-di-GMP synthesis or hydrolysis. But why zinc?

To answer this question, Zähringer et al. (2013) examined the physiological role of DgcZ. This DGC has been shown to stimulate synthesis of the exopolysaccharide, poly-β-N-acetylglucosamine, a major biofilm component in *E. coli* and other bacteria (Römling et al., 2013). In vivo, biofilm formation was found to be modulated by the concentration of Zn^{2+} in the medium, and Zn^{2+} dependent biofilms were shown to require DgcZ and its product, c-di-GMP. Therefore, DgcZ operates as a Zn^{2+} sensor that controls *E. coli* biofilm formation. Where does *E. coli* face environments with shortages

or excesses of Zn^{2+}? Apparently different natural environments have varying Zn^{2+} concentrations. Another possibility is that sensing Zn^{2+} may be important inside animal bodies, where available Zn^{2+} levels differ by several orders of magnitude. Furthermore, starving microbes for Zn^{2+} and poisoning them by the excess of Zn^{2+} are both known strategies employed by animal systems to fight microbial infections (Hood and Skaar, 2012).

Light, oxygen, NO, protein phosphorylation, and now Zn^{2+} has been added to the growing number of signals regulating DGCs. Along with the growing knowledge of signals affecting DGCs, our understanding of how bacteria adjust their lifestyles to diverse environments and how we can manipulate these environments to control bacteria grows. The structural insights from this work reinforce the impression from earlier structural studies (Paul et al., 2007; De et al., 2009) that proper positioning of two rigid GGDEF domains in a homodimer is necessary and sufficient for sparking DGC activity. A direct consequence of this realization is that GGDEF domains in most (if not all) DGCs may be replaced with heterologous GGDEF domains with stronger or weaker activities; thus, one may be able to engineer DGCs with varying potencies to be regulated by desired signals. Another corollary is that sensing modules controlling mutual positioning of the GGDEF monomers may be employed to regulate heterologous protein domains if homodimerization of these domains is sufficient for activation, a feat recently accomplished by replacing a GGDEF domain with an adenylate cyclase domain (Gomelsky and Ryu, 2012).

Lastly, it is with great sadness that one realizes that we may no longer enjoy beautiful pieces of work like this study to be authored by the senior author, Alex Boehm, because his scientific career has been abruptly brought to a halt by an aggressive cancer (Boos et al., 2013).

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Decoding a Chain Letter for Degradation

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Ubiquitin chains can have distinct signaling outcomes, depending on their conjugation point. In this issue of *Structure*, Castañeda and colleagues describe a new structure of K11 diubiquitin and investigate its recognition by effectors to target substrates to the proteasome.

Protein ubiquitination is an essential post-translational modification that is integral to many cellular processes, including protein stability, localization, cell signaling, and cell cycle regulation (Hershko and Ciechanover, 1998). Ubiquitination minimally consists of a single ubiquitin linked to an accessible lysine on a target protein via a C-terminal isopeptide bond. However, ubiquitin can also form chains on target proteins through self-conjugation on any of its seven lysine residues or its N-terminus (Komander and Rape, 2012). These polyubiquitin chains may contain homotypic or heterotypic linkages, implying a vast potential of information content for cellular signaling.

Several studies in recent years have demonstrated that each ubiquitin linkage type encodes a different chain structure. K48-linked polyubiquitin forms a compact structure stabilized by an extensive hydrophobic interaction surface centered around Ile44, a major interaction motif for ubiquitin binding proteins (Komander and Rape, 2012). A distinct compact conformation was also observed in multiple structures of K6 diubiquitin (Hospenthal et al., 2013; Virdee et al., 2010). In contrast, there are no inter-ubiquitin interaction surfaces observed in K63-linked or linear chains, which form extended conformations (Komander and Rape, 2012). Each distinct chain conformation exposes different surfaces to ubiquitin binding partners, which in turn confer

